Peer Review
Experimental Toxicology Division
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Cellular Metabolism and Toxic Effects of Arsenicals

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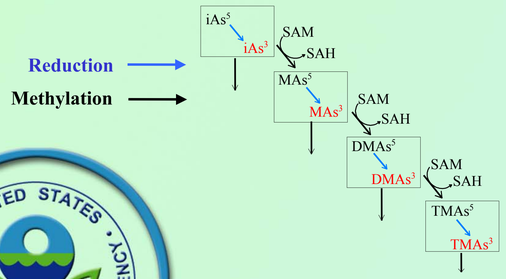
Introduction

Chronic exposure to inorganic arsenic (iAs) has been associated with increased risk of a variety of adverse health effects. Two broad issues have been raised in the risk assessment for iAs. First, what is the nature of the dose-response relationship between iAs exposure and the development of specific adverse health effects? Second, by what molecular mechanisms does iAs exert its deleterious effects? Research in ETD provides new insights into both dose-response relationships and mode of action for iAs.

Work in this laboratory has shown that enzymatically-catalyzed methylation of iAs yields products that exceed iAs in reactivity and toxicity. Of special interest are the methylated arsenicals that contain trivalent arsenic (methylarsonous (AsIII) acid and dimethylarsinous (AsIII) acid) which are produced in cellular metabolism of iAs (see scheme below).

Two general questions have been addressed in this work:

- Do the putative intermediates in the pathway for iAs adversely affect cellular functions?
- Can we detect these intermediates in cellular environments?



Scheme for cellular metabolism of inorganic arsenic.

Methods

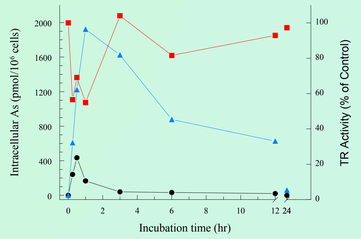
Cell lines - The metabolism of arsenicals and their effects on cellular function have been studied in a variety of cell lines. These include primary rat and human hepatocytes, and UROtsa cells, a human urinary bladder epithelial cell line. Both human and rat hepatocytes rapidly metabolize inorganic arsenic. UROtsa cells do not express cyt19 mRNA and do not methylate inorganic arsenic.

Arsenic analyses - The following methods were used in these studies.

- Determination of radiolabeled arsenicals by thin layer chromatography.
- Determination of speciated arsenicals in biological samples by hydride generation-atomic absorption spectrometry. This method has been adapted for permit the selective detection of arsenicals containing pentavalent or trivalent arsenic.

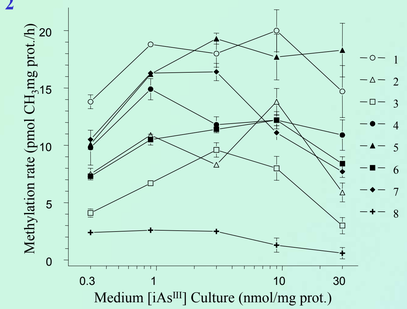
Results

1



In cultured primary rat hepatocytes exposed to inorganic arsenic, the inhibition of thioredoxin reductase activity (■) is maximal when the intracellular concentration of methyl arsenic (●) is maximal. High cellular concentrations of dimethyl arsenic (▲) are not associated with inhibition of thioredoxin activity.

2



Concentration dependence of the rate of arsenic methylation for primary human hepatocytes exposed to inorganic arsenic (III). Data shown for 8 donors.

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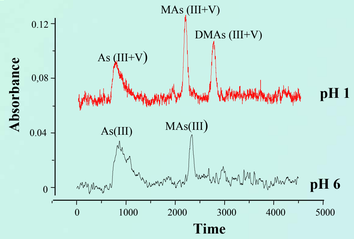

Formation of trivalent methylated arsenicals in primary human hepatocytes from a single donor exposed to 0.1 μM inorganic arsenic (III) for 24 hours. Cell lysate prepared for analysis by pH selective hydride generation-atomic absorption spectrometry. Cells contain methylated arsenicals with both trivalent and pentavalent arsenic.

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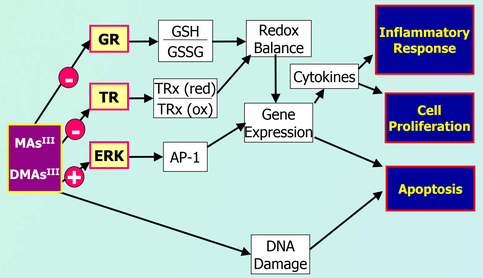

Scheme summarizing the effects of methylated arsenicals containing trivalent arsenic on cellular functions. Here, metabolites of inorganic arsenic potently inhibit the activities of glutathione reductase (GR) and thioredoxin reductase (TR). Altered activity of these enzymes affects the ratio of glutathione (GSH) to GSH disulfide (GSSG) or of reduced and oxidized thioredoxin (Trx). This shift affects the redox balance in the cell, triggering expression of genes controlling cytokine levels and of genes involved in apoptosis. Alterations of gene expression by arsenicals provokes an inflammatory response, increases cell proliferation, or stimulates programmed cell death. Alternately, the methylated metabolites also activate ERK, increasing levels of the AP-1 transcription factor and altering gene expression. In addition, there is evidence that the metabolites also damage DNA, possibly through formation of reactive oxygen species.

Conclusions

Inorganic arsenic is rapidly converted to methylated species in cultured cells. The presence of these methylated species in cells is associated with perturbations of cellular function. For instance, the transient inhibition of thioredoxin reductase activity in cultured rat hepatocytes is associated with an increased cell content of methylated arsenic. There are striking differences in the capacity of cultured human hepatocytes to form the methylated arsenicals. This suggests that differences in response to arsenic exposure could be partly determined by interindividual differences in the capacity to methylate arsenic. Such interindividual differences could also affect the capacity to form highly reactive methylated arsenicals that contain trivalent arsenic. Thus, it appears that putative intermediates in the pathway for arsenic metabolism are present in cellular environments and these metabolites can adversely affect cellular functions.

Impact

These results confirm the importance of enzymatically-catalyzed methylation of inorganic arsenic as a determinant of the toxicity of arsenic. Evidence for interindividual variability in the levels of these metabolites in cells draws attention to the potential role of interindividual variation in metabolism in interindividual variation in susceptibility to the adverse effects of arsenic exposure.

Future Directions

Additional studies will elucidate the linkage between the capacity of cells to metabolize arsenic and the many effects that arsenic exposure exerts on cell function.

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